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EXPERIMENTS ON THE EXTRUSION OF POLAR FILAMENTS OF CNIDOSPORIDIAN SPORES *

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It has been generally believed that when a Cnidosporidian spore is introduced into the digestive tract of a new host, the first change of the spore prior to the germination of the sporoplasm is the extrusion of its polar filament, according to the observations made by Thélohan (1895), Stempell (1909), Fantham and Porter (1912), Kudo (1916), and others. Osmotic pressure is considered in this case to be the cause of the filament extrusion.

In the case of the artificial cultivation of the Cnidosporidia, starting with the spore-stage, therefore, the first observation should be concentrated on the filament. From this point of view I have again taken up the question of the filament-extrusion, which I partially worked out about four years ago (Kudo, 1913).

Since Balbiani discovered in some Myxosporidian spores the presence of a polar filament which was extruded from the spore under the action of alkalies, several investigators have tried a large number of reagents in the effort to produce extrusion of the filament of Cnidosporidian spores of various species. The reagents used and the authors' names are given in Table 1.

Most authors agree that the percentage of spores which extrude the filament when subjected to the action of the above-mentioned reagents is very small and irregular. It seems quite remarkable that these reagents should affect only certain particular species, as would appear from Thélohan's experiment (1895).

In the case of mechanical pressure, I could see almost all of the spores extruding their filaments at places where the pressure was apparently strong enough to drive the filament out. When the spore material is mixed with comparatively coarse particles of tissue, however, the pressure method does not give uniform results.

* Work carried out in the laboratories of Dr. Noguchi and under his direction.

Frey and Lebert and Haberlandt and Verson studied the action of several chemicals upon the spore of *Nosema bombycis* without noticing the filament extrusion.

Of the many chemicals which I have tried up to the present, hydrogen peroxid, as first found by Dr. Noguchi, has proved best. Following is a brief summary of the experiments with this reagent, which have been carried out chiefly on *Nosema bombycis*.

TABLE 1

Reagents	Author's Name and Parasite Used
Acetic acid	Thélohan (*Myxosoma, Henneguya*), Wasielewski, Fantham and Porter (*Nosema apis*)
Ether	Thélohan (*Thelohania*) Gurley, Pfeiffer, Wasielewski, Auerbach
Alkalies (KOH and NaOH)	Balbiani, Thélohan, Wasielewski, Auerbach, Kudo
Ammonia	Auerbach, Schuberg (*Pleistophora longifilis*)
Boiling water	Gurley, Wasielewski, Auerbach
Distilled water	Thélohan (*Chloromyxum*), Wasielewski, Auerbach
Glycerin	Schneider, Gurley, Wasielewski, Auerbach, Awerinzew (*Myxobolus magnus*)
Iodin alcohol	Stempell (*Thelohania mülleri, Nosema anomalum*)
Iodin water	Thélohan (*Ceratomyxa, Glugea, Pleistophora*), Léger, Wasielewski, Auerbach, Fantham and Porter
Mineral acids:	
Hydrochloric acid	Thélohan (*Thelohania*), Gurley, Wasielewski, Auerbach
Nitric acid	Thélohan (*Myxospordia, Glugea bombycis, Thelohania*), Gurley, Pfeiffer (*Thelohania mülleri*), Wasielewski
Sulphuric acid	Bütschli, Thélohan (*Sphaeromyxa*), Gurley, Wasielewski, Auerbach, Awerinzew
Physiological solution	Lacépède (*Pleistophora macrospora*)
Pressure	Gurley, Auerbach, Kudo

The sterile silk-glands taken from the highly infected larvae of *Bombyx mori* furnished the original material for the experiment. They were emulsified with sterile water as well as with sterile Ringer's solution, kept in small test tubes in the refrigerator at a temperature of 6° C., and taken out from time to time for examination. An equal quantity of a strong solution of hydrogen peroxid and the emulsion were mixed either on a slide or in a test tube and examined microscopically. Observations were usually made under the dark field microscope, which, though it seems to have been neglected in this field of protozoology, affords a quick and entirely suitable means of carrying out the necessary observations. The permanent preparation was made according to Löffler—Kudo's method (1913). Fontana's staining for *Treponema pallidum*, however, was found to be equally good.

When a drop of the emulsion of *Nosema bombycis* is mixed on an acid-free slide with a drop of perhydrol,* active bubbling takes place. By examining the preparation under the dark-field microscope, almost all of the spores are seen to shoot out the polar filament with great rapidity (see plate). The phenomenon takes place immediately and lasts for several minutes.

The emulsion in Ringer's solution seems to give a more vigorous extrusion than the plain water emulsion.

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* About 30% H_2O_2 by weight, Merck.

A weak alkalin reaction of the emulsion as rendered by the addition of sodium bicarbonate seems to accelerate the extrusion of filaments, while acidulation inhibits extrusion.

That a concentrated solution of perhydrol is much more effective than a weaker one is shown in Table 4.

An attempt was made to induce the extrusion of polar filaments with the emulsions prepared from old dried materials, but it was found that no extrusion took place with such specimens. Apparently the desiccation of the spores rendered them insensitive to the extruding action of the reagent. The results of the experiments made with freshly dried materials were identical with those obtained with the old specimens and are shown in Table 5. An electric fan was employed to dry the emulsion during the first 24 hours, after which the slides with the dried emulsions were left at room temperature.

TABLE 2

	Immediately After Treatment	After One Hour	After 24 Hours
Water emulsion, 1 c.c. + perhydrol, 2 c.c.	Many extruded filaments	Fairly many extrusions	Many filaments detached
Ringer emulsion, 1 c.c., + perhydrol, 2 c.c.	Almost all spores show filament	Almost all spores show filament	Many filaments detached

TABLE 3

	Control	$NaHCO_3$ (1%)	HCl (1%)
Ringer emulsion, 1 c.c., + perhydrol, 2 c.c.	Many filaments extruded	Almost all spores show extruded filament	No extrusion

TABLE 4

Ringer emulsion, 1 c.c. + perhydrol (30%), 1 c.c.	Immediately after the treatment great many extruded filaments
Ringer emulsion, 1 c.c. + perhydrol solution (3%), 1 c.c.	A few extruded filaments

TABLE 5

	Perhydrol and spores in alkalinized emulsion	1% aqueous emulsion of the fresh tissue, $NaHCO_3$ and perhydrol	Spores taken from dried moths, which were kept over one year, pressure method
Immediately after the aqueous emulsion was made	Almost all of the spores show extruding filaments	Vigorous bubbling	Many filaments
Dried for 2 hours on slide	Fairly many extruded filaments	Bubbling less active	
Dried for 16 hours on slide	Many extruded filaments	Weak bubbling	
Dried for 24 hours on slide	A few detached filaments (?)	Only few bubbles	
Dried for 3 days on slide	No extrusion	No bubbling nor bubbles	
Dried for 5 days on slide	No extrusion	No bubbling nor bubbles	
Dried for 10 days on slide	No extrusion	No bubbling nor bubbles	

It is noteworthy that the number of extruded filaments progressively diminished as the desiccation went on, until, after three days, the phenomenon ceased to occur. With regard to the mechanism of extrusion by means of perhydrol, it seems probable that a physical force, comparable to that produced by pressure, develops within the spores and expels the polar filament. This force may be none other

than the gas evolved through the decomposition of hydrogen peroxid by the peroxydase contained within the spores. In support of this view it may be cited that the spores preserved for more than one year still extruded their filaments when subjected to mechanical pressure. The failure of the dried spores, therefore, to extrude the polar filament under the action of perhydrol is a sign of the weakening or absence of the peroxydase, but bears no relation to the viability of such spores.

TABLE 6

	Perhydrol + spores in alkalinized emulsion	Pressure
1 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
3 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
10 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
30 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
60 per cent. methyl alcohol	Few extruded filaments....	Few extruded filaments
70 per cent. methyl alcohol	Few detached filaments (?).	?
85 per cent. methyl alcohol	No extrusion	No extrusion
Absolute methyl alcohol...	No extrusion	No extrusion
Control without alcohol treatment	Almost all spores show extruded filament	Many extruded filaments

TABLE 7

	Perhydrol + spores in alkalanized emulsion	Pressure
10% ethyl alcohol, 16 hours	Few extruded filaments.....	Many extruded filaments
30% ethyl alcohol, 16 hours	Few extruded filaments.....	Few extruded filaments
34% ethyl alcohol, 16 hours	Few extruded filaments.....	?
38% ethyl alcohol, 16 hours	No extruded filaments......	?
50% ethyl alcohol, 16 hours	No extruded filaments......	No extruded filaments
80% ethyl alcohol, 16 hours	No extruded filaments......	No extruded filametns
Absolute methyl alcohol...	No extruded filaments......	No extruded filametns
Control without alcohol treatment	Almost all spores show extruded filament	Extruded filaments in many places

TABLE 8

	Ethyl Alcohol		Methyl Alcohol	
	$+H_2O_2$ (0.02 C.c.)	+1% $NaHCO_3$ (0.5 C.c.) $+H_2O_2$ (0.02 C.c.)	$+H_2O_2$ (0.02 C.c.)	+1% $NaHCO_3$ (0.5 C.c.) $+H_2O_2$ (0.02 C.c.)
Control without alcoholic treatment	Vigorous bubbling	Vigorous bubbling	Vigorous bubbling	Vigorous bubbling
Emulsion +10%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +20%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +30%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +40%	Active bubbling	Active bubbling	Less active bubbling	Active bubbling
Emulsion +50%	Active bubbling	Active bubbling	Less active bubbling	Active bubbling
Emulsion +60%	Less active bubbling	Active bubbling	No bubbling	Less active bubbling
Emulsion +70%	Less active bubbling	Active bubbling	No bubbling	Much less active bubbling
Emulsion +80%	No bubbling	Less active bubbling	No bubbling	No bubbling
Emulsion +90%	No bubbling	No bubbling	No bubbling	No bubbling
Emulsion + absolute	No bubbling	No bubbling	No bubbling	No bubbling

It is evident that in the case of perhydrol, the extrusion of filaments is produced by a positive pressure created within the spores, but it was not shown whether or not a negative pressure by means of a vacuum will accomplish the same effect. For this reason, fresh Nosema spores were placed in a vacuum jar from which the air was exhausted with an electric vacuum pump. A vacuum at 2 mm. of mercury maintained for one hour did not expel the polar filaments from the spores.

With reference to the effect of alcohol upon the extrusion of polar filaments, it may be remarked here that Thélohan (1895) working with various chemicals as already quoted in the earlier part of this article, failed to obtain any extrusion with several Myxosporidia preserved in alcohol. On the other hand, Gurley (1894) reports that a rather small proportion of the spores of certain Myxosporidia preserved in alcohol extruded the polar filament under the action both of sulphuric acid and iodin water.

An experiment bearing on this point was made in the present study with *Nosema bombycis*. The technic used to test the action of alcohol upon the Nosema spores consisted in mixing 0.5 c.c. of a water emulsion and 3 c.c. of each of several alcohols in test tubes and allowing the mixtures to stand for ten minutes, inclusive of the time required for centrifugation, or, in some instances, for 16 hours at room temperature before centrifugation. The spores deposited at the bottom of the centrifuge tubes were subjected to the action of perhydrol in concentrated form. The results are recorded in Table 6 and 7.

As will be noted in the foregoing tables, of the spores subjected to the action of 60 per cent. methyl alcohol for 10 minutes, or of 34 per cent. ethyl alcohol for 16 hours, a few still extrude their polar filaments when treated with perhydrol. No polar filament is seen to be extruded from the spores treated with 85 per cent. methyl alcohol for 10 minutes or 30 per cent. ethyl alcohol for 16 hours. Methyl alcohol weaker than 30 per cent. allowed to act for 10 minutes had much less effect upon the phenomenon. Spores which have remained in 30 per cent. ethyl alcohol for sixteen hours were rendered almost insusceptible to the extruding action of perhydrol, and the filament extrusion became less as the percentage of alcohols rose, until, when the spores were treated with strong alcohol for a sufficient length of time, no filament escaped from the spore. In this instance, as in that of desiccation, the reduction of susceptibility of these spores to the extruding action of perhydrol may be explained by assumptions similar to those advanced for the effect of desiccation. Alcohol may have altered the permeability of the spores, or possibly the elasticity of the polar filament is reduced by the hardening action of alcohols, rendering it unable to unfold for protrusion. Another factor is the gradual destruction of the peroxydase as the concentration of alcohols is increased. Table 8 records the results of an experiment which shows that the peroxydase is considerably damaged by 30 to 40 per cent. methyl alcohol or 50 to 60 per cent. ethyl alcohol when acted upon for three hours. Thus 0.05 gm. of a fresh rabbit's kidney was emulsified with 5 c.c. of distilled water; 0.05 c.c. of the emulsion was

mixed with 0.5 c.c. of each of several grades of alcohol, and the mixtures were left in the refrigerator at 6° C. for three hours. At the end of this period they were further treated with perhydrol (0.02 c.c.), or with 1 per cent. $NaHCO_3$ (0.05 c.c.) and perhydrol (0.02 c.c.). The results were as follows:

It may be concluded, therefore, that a fresh tissue treated with 90 per cent. ethyl alcohol or 80 per cent. methyl alcohol is no longer able to split hydrogen peroxid under the experimental conditions described above. Assuming that the extrusion of polar filaments is caused by the decomposition of H_2O_2 by a minute amount of the intracellular ferment (peroxydase), it is not difficult to understand why the extrusion becomes less certain when desiccation or alcohol treatment is applied to the spores, since there results an inactivation of the ferment sufficient to cause the occurrence of the phenomenon.

Spores of several species of Myxosporidia were also subjected to the action of perhydrol. The following Myxosporidian spores, according to my observations (Kudo, 1916a) when treated with this reagent, extrude their filaments: *Myxidium* sp.; *Zschokkeella acheilognathi* Kudo; *Myxosoma funduli* Kudo (Fig. 6).

SUMMARY

1. A concentrated solution of hydrogen peroxid is the most perfect and convenient reagent for producing extrusion of the polar filament from spores of *Nosema bombycis* and of some Myxosporidia in the fresh state.

2. The action of hydrogen peroxid is accelerated by the presence of weak alkalies.

3. Ringer's solution emulsion is more favorable for filament extrusion than water emulsion.

4. The action of hydrogen peroxid in extruding the polar filament is less effective upon spores which have been desiccated at room temperature than upon fresh ones. Spores dried on a slide for three days do not extrude the filament.

5. The pressure method gives, generally speaking, the same results as the perhydrol method, except that it produces fewer examples of extruded filament.

6. A spore emulsion centrifuged with 60 per cent. methyl alcohol for 10 minutes or mixed with 34 per cent. ethyl alcohol for 16 hours shows filament extrusion under the action of perhydrol.

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TWO NEW CYSTOCERCOUS CERCARIAE FROM NORTH AMERICA*

Ernest Carroll Faust

Following the description of the unique anchor-tailed *Cercaria mirabilis* by Braun (1891), Ward (1916) published an account of two similar American cystocercous species, *Cercaria wrightii* and *C. anchoroides*. The former of these American species had been previously mentioned by Wright (1885) and Leuckart (1886). Knowledge of the structure and relationship of this group is further augmented by the study of two new American species, for which the names *Cercaria brookoveri* and *C. macrostoma* are proposed.

Cercaria brookoveri nov. spec.

This interesting cystocercous cercaria was obtained by Professor Chas. Brookover from Campeloma sp. at Cedar Point, Lake Erie, April 23, 1912. The writer has been enabled to examine it thru the kindness of Professor Henry B. Ward. The worm consists of a body of typical distome characters, 0.52 to 0.62 mm. long and 0.3 to 0.37 mm. wide, a large sac-like tail trunk 1.37 mm. long and 0.46 mm. in diameter, and a pair of bluntly pointed anchor-flaps 0.45 mm. long and 0.35 mm. wide. The distome is not included within the anterior region of the tail trunk as in the previously described cercariae of this type (*C. mirabilis, C. wrightii, C. anchoroides*), but is attached at its posterior end to the trunk by a stipe, and surrounded for a short distance by a collar extension of the trunk. A cross section of the tail trunk is circular with a large central lumen, the caudal excretory trunk, while a matrix of loosely woven parenchyma cells fills the interstices between lumen and ectoderm. The excretory tube divides anterior to the anchor flaps, one branch entering each flap.

The distome is round to oval in cross section. The oral sucker, 40 by 38μ, is directed ventrad. The acetabulum, 41 by 32μ, is slightly anterior to the middle of the body. Above the oral sucker and slightly caudad is the pharynx, 21μ in diameter. It leads into the intestinal ceca thru a very short esophagus. The diverticula extend laterad, then caudad, toward the posterior margin of the worm. They are somewhat convoluted and are filled with a semi-transparent jelly mass, which includes many small refractive granules and vacuoles. The

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walls of the diverticula consist of flat polygonal cells, scarcely differentiated from the body parenchyma. The excretory bladder is posterior. From it a large median tube leads forward toward the acetabulum. Its further course has not been observed. The reproductive organs are represented by a large oval cell mass posterior to the acetabulum, an elongate mass under the anterior wall of the acetabulum and a chord connecting these two masses around the right wall of the acetabulum. A comparison of size measurements of *C. brookoveri* and *C. anchoroides* seems to indicate that the two species are distinct (see table).

The nervous system is plainly detailed in frontal sections of the cercaria. There is a small cerebral ganglion mass and a subesophageal commissure almost as large. The ventrales and dorsales are easily found. Posterior to the brain the ventrales are the most conspicuous. From the subesophageal commissure two pharyngeales with intercommunicating network are derived. Along the ventral trunks the preacetabular and postacetabular commissure are prominent. There are many ventral commissures.

Like *Cercaria mirabilis* (Braun 1891) this cercaria develops within a sporocyst. The sporocyst of *C. brookoveri* reaches a length of 2.6 mm. and a cross diameter of 0.9 mm. Within it are all stages of developing cercariae from germ ball to mature larva, but only one individual of each stage is found at any one time (Figs. 2, 3, 5). The sporocyst is elongate to oval and has many prominent muscular annuli around its outer girth. As the cercaria develops the distome body is at first largest and most distinctive, with only a suggestion of the anchor flaps (Fig. 3). Later, however, the tail trunk elongates and the junction of body and tail becomes more differentiated by the formation of a stipe connection and a partial enveloping of the posterior part of the worm with the anterior part of the tail trunk. The mature larva differs from this stage in the amplification of the main trunk of the tail and the differentiation of the anchor flaps.

There is evidence to show that *C. brookoveri* is cannibalistic. Within a sporocyst of this species the larger and more mature larvae with their powerful suckers secure a hold on the less mature individuals and, with the aid of secretory juices, digest them bit by bit (Fig. 6). Thus it is probable that many germ balls are produced which never are allowed to mature because they are ingested by their more hardy congeners.

Cercaria macrostoma nov. spec.

A single specimen of this larva for which the name *Cercaria macrostoma* is proposed was found in an aquarium in the Zoological Laboratory of the University of Illinois in October, 1917. The writer

desires to thank Mr. E. C. Harrah for the specimen. The aquarium had been used for a week to contain several specimens of *Campeloma subsolidum* (Anthony) and *Goniobasis pulchella* (Anthony) before the fluke was discovered. The size of the larva and the paddle movement of the tail flaps, together with the creamy yellow color of the whole body, made the worm a conspicuous object. The movement was similar to that described by Ward (1916) for *C. anchoroides*. At first only the superficial features of the larva were evident, but under pressure of the cover slip the internal characters became clear. The trunk is a thick, oblong object, slightly conical anteriad and wedge-shaped posteriad. The flappers are broadly spatulate. At times they are found at right angles to the trunk, at other times they approximate one another as the wings of a butterfly. At the anterior end of the trunk there is a central median evertible proboscis surrounded by a ring of five large wartose papillae. Two of these are ventral, two lateral, and one median dorsal. Spread over the anterior two-thirds of the trunk are irregular rings of warts somewhat smaller than those around the anterior end. The larval distome is found within the anterior two-fifths of the enveloping trunk.

Cercaria macrostoma has a measurement of 5.0 mm. for the caudal trunk length and a cross diameter of 1.1 mm. Thus it exceeds *C. wrightii*, *C. anchoroides* and *C. brookoveri* in size and approximates *C. mirabilis* (Braun 1891). The flappers are 1.2 mm. long and 0.83 wide. The distome itself measures 1.2 mm. in length by 0.5 mm. in width. It is broadest near the posterior end.

The oral sucker of the larval distome is large, measuring 0.48 by 0.36 mm. in diameter, while the acetabulum is 0.20 mm. wide and 0.26 mm. long. The latter is situated somewhat posterior to the middle of the body. The oral sucker leads into a pharynx 70μ in diameter. The esophagus is very short. The ceca first run dorsad then laterad, and then continue posteriad to the caudal region of the body. Here they curve inward and almost meet in the region ventral to the excretory bladder. They are coiled thruout their entire length. They are filled with a semitransparent yellow jelly mass in which are imbedded refractive granules. The excretory system is constructed on the plan of a long slender Y, with a slightly dilated base. The fork occurs just dorsal to the acetabulum. One pair of fine capillaries extends inward in the region of the pharynx, but the main tubule extends forward on each side around the oral sucker.

The reproductive organs are well developed, constituting a precocious condition (Fig. 7). A pair of testes and an ovary are found behind the acetabulum. In front of the acetabulum is a large cirrus pouch with thick cuticular walls and further dorsad is a large seminal

vesicle. A uterus filled with rips eggs is found to arise in the region of the ovary, and, after coiling a short distance backward, is seen to turn to the right of the acetabulum and run to the region of the pharynx, and thence to the genital pore. The eggs measure 78 to 88μ in length by 47 to 50μ in cross diameter. The vitelline follicles are arranged in two loosely strung chords at the sides of the body. They extend from the region of the pharynx to the posterior region of the body. This fluke is probably an Allocreadiine species.

TABLE OF COMPARATIVE DATA ON CYSTOCERCOUS CERCARIAE

	C. mirabilis Braun	*C. wrightii* Ward	*C. anchoroides* Ward	*C. brookoveri* Faust	*C. macrostoma* Faust
Found	Free, aquarium	Free, aquarium	Free, Lake St. Clair	In snail	Free, aquarium
Place	Kurland	Toronto, Can.		Sandusky, O.	Urbana, Ill.
Date	1891	1885	1893	1912	1917
Larval host..	*Lymnaea palustris corvus*	Unknown	Unknown	Campeloma sp.	Unknown
Parthenita ...	Sporocyst	....	....	Sporocyst	....
Size Distome					
Length	....	0.45 mm.	0.64 mm.	0.52-0.62 mm.	1.2 mm.
Width	....	0.1 mm.	0.288 mm.	0.3-0.37 mm.	0.5 mm.
Tail					
Length	6.0 mm.	1.0 mm.	2.0 mm.	1.37 mm.	5.0 mm.
Width	....	0.133 mm.	0.28 mm.	0.46 mm.	1.1 mm.
Flappers					
Length	1.5 mm.	0.533 mm.	0.53-0.6 mm.	0.45 mm.	1.2 mm.
Width	....	0.1 mm.	0.24-0.34 mm.	0.35 mm.	0.83 mm.
Suckers					
Oral	....	41μ	160μ	40x38μ	480x360μ
Ventral ...	Larger than oral	75μ	128-144μ	41x32μ	200x260μ
Digestive System					
Ceca	....	....	Large, heavy	Large	Crowded, especially in posterior end
Pharynx					
Diameter ..	....	....	64μ	21μ	70μ
Excretory System ..	....	....	Reservoir reaches acetabulum	Reservoir ends little posterior to acetabulum	Reservoir continues anterior to acetabulum

GENERAL CONSIDERATIONS

Aside from their anchor tail the species of this group possess other characters in common which demonstrate their close relationship. Among these are the crowded ceca with granular contents, the long median Y-shaped excretory bladder, the presence of the ovary and pair of testes close behind the acetabulum, and the swollen cirrus pouch anterior to the acetabulum. It seems significant, likewise, that Braun (1891) and Ward (1916) found wart-like protuberances on the external surfaces of the trunk, similar to those recorded for *C. macrostoma*. These species differ from *Cercaria macrocerca* de Filippi in possessing no stylet. The latter species also has no anchor flaps to the tail.

On account of the fundamental agreement of the five described species of this group, the common history of all may be learned by coordinating data from the several species. In general, the cercariae develop as the parthenogenetic offspring of sporocysts. They are found in the respiratory or digestive organs of snails. In the differentiation of the germ balls the suckers first become set off from the body, then the caudal organ is outlined. The anchor flaps first show as stubs. Somewhat later the region between body and tail becomes differentiated into a central stipe and an outer enveloping collar. In *C. brookoveri,* perhaps the least modified of all the described species, the collar is only a partial envelop. In the other four species, however, this portion of the trunk comes to surround the worm entirely save for a pore at the anterior end of the animal. Further differentiation consists in the formation of warts at various places, but most prominently around the anterior end of the worm. In *C. macrostoma* there are, in addition to the ordinary warts, five prominent papillae around the oral opening of the cyst. Ward (1916: 16) has noted that the movement of the worm is the reverse of that usually found in other groups of cercariae.

While the digestive and excretory organs are equally developed in all of the species, the genital system in *C. macrostoma* alone are sufficiently mature to warrant a suggestion of the systematic position of the group. These quite definitely relate the group to the Allocreadiidae. The limited extent of the uterus excludes these species from the Bunoderinae. The papillae on the outer surface of the worms are structurally similar to those in the Stephanophialinae, but are located on the modified portion of the tail rather than on the body of the flukes, hence are not to be considered as homologous to the oral papillae of the Stephanophialinae. The only Allocread species, the life history of which is known, is *Allocreadium isoporum* Looss. The larva of this species has a rhopalocercous structure, which is somewhat simpler than that of *C. brookoveri.* When all of the group characters are considered it seems highly probable that these five species represent a portion of the Allocreadiinae, for which there is as yet no satisfactory classification.

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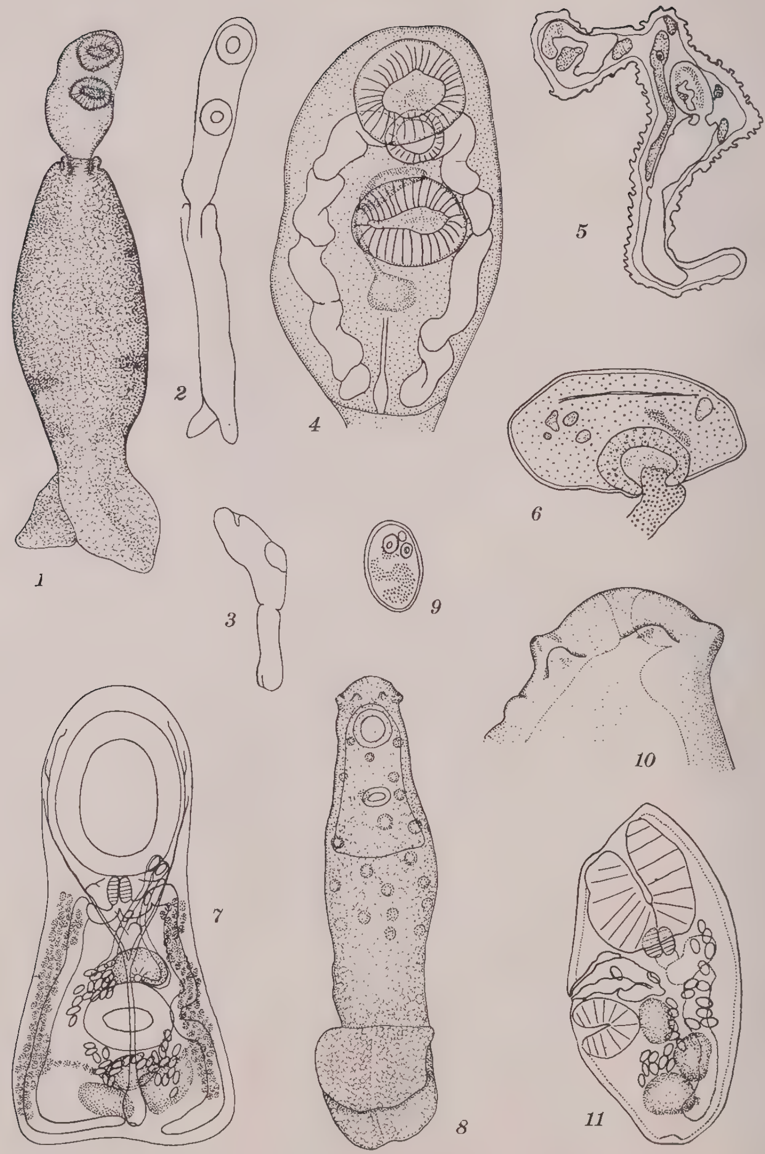

PLATE II

EXPLANATION OF PLATE

Cercaria brookoveri. 1. Sketch of entire cercaria, ventral view, × 34. 2, 3. Stages in the development of the cercaria, × 54. 4. Body of the worm enlarged, ventral view, × 105. 5. Section of sporocyst with developing cercariae, × 34. 6. Section of a maturing individual ingesting a younger individual, × 75.

Cercaria macrostoma. 7. Ventral view of living fluke compressed, showing important details of digestive, excretory and genital systems, × 54. 8. Sketch of entire cercaria, ventral view, × 14. 9. Uterine egg, × 170. 10. Detail of anterior end of cyst, showing papillae, lateral view, ×26. 11. Lateral view of preserved fluke, × 34.

THE TICK AS A POSSIBLE AGENT IN THE COLLOCATION OF THE EGGS OF *DERMATOBIA HOMINIS* *

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Sufficient observations, consisting of more or less convincing facts and an abundance of circumstantial evidence relating to the egg disposal of *Dermatobia hominis,* have been reported by different writers to prove definitely that a certain species of mosquito, *Psorophora lutzii,* is the active agent in this disposal. However, neither these facts or other evidence that have been submitted up to date are concrete enough to prove that this mosquito is the sole carrier.

In view of this doubt which still reasonably exists in regard to other insects also acting as vectors, one case of infestation with the larvae of this fly was recently brought to the attention of the writer that is considered sufficiently interesting to be worthy of report.

In February of the present year, two members of the staff of this laboratory, Dr. H. C. Clark, pathologist, and Mr. J. E. Jacob, chemist, accompanied by Mrs. Clark, left for a few weeks hunting trip near the headwaters of the Boqueron River in the interior of Panama. Aside from hunting it was also intended to make more or less of a survey of the diseases common among both the human and animal inhabitants of that region. Specimens of insects were also to be collected, especially of the groups known to be concerned in the transmission of the various insect borne diseases of man and animals.

As this was in the dry season of the year the ticks were very numerous and proved to be exceedingly troublesome pests. After being in this region about nine days, Dr. Clark, upon returning to camp after a day's hunt, on February 16, found an adult tick firmly attached to the back of his left hand. It was an unengorged female and had attached itself near the wrist joint on the median line of the hand. The locaton was at the point where a shirt or coat sleeve usually touches the hand. After the removal of the tick the site of attachment was painted with tincture of iodin followed by alcohol.

Two days later upon returning to camp, Dr. Clark found that he was again acting as a host for ticks, and that this time there were three. All three were firmly attached on portions of the body that

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* Read before the Medical Association of the Isthmian Canal Zone, January 18, 1918.

were well protected with clothing. Two were located on the upper left side of the abdomen at a point above the belt line. They were attached about two inches apart. The third tick was attached on the left side of the back, also above the belt line. All three were removed and the site of each attachment well swabbed with iodin.

The four sites from which these three ticks, and the one previously found on the back of the hand, had been removed behaved like tick bites in which the proboscis had been left in the skin and delayed the healing. The tiny wounds refused to heal, although all four were treated twice a day with iodin followed by alcohol. The iodin was even introduced well down into the wound on the sharpened end of a match. Each of the four small wounds remained unchanged and continued to ooze small quantities of serum at intervals without any healing results being obtained from the iodin treatment, until February 27. On this date while returning to the Canal Zone, Dr. Clark noticed the lesion on the left hand beginning to swell like a small boil, and it soon became rather painful. This may have been hastened by using the hand rather freely in paddling a canoe. It had been suspected for several days that the presence of a larva in the wound was the cause of the non-healing. An examination was now made with the aid of a hand lens which verified the suspicion and an incision produced a Dermatobia larva 4 mm. in length.

Although it was now quite certain that the three lesions on the body also contained larvae, no further attention was given them except to continue the iodin treatment daily. They remained unchanged until March 6, when an area of from one-half to one and one-half inches around each wound began to swell and become red and painful. Frequent pricking and boring sensations were felt in the wounds, which at times were equivalent to the pricking of a hypodermic needle. A serous exudation was also occasionally noticed. This continued until March 8, when quite a severe glandular swelling began to take place on the left side, and it was then considered advisable to remove the larvae. Incisions were now made and a small Dermatobia larva removed from each wound. The smallest one was 2.5 mm. in length, and the largest one approximately 4 mm.

In order to make a positive identification of the larvae, when the first one was removed from the back of the hand it was placed in a small incision made in the skin on the back of the neck of a guinea-pig. This was done within an hour after its removal. It immediately began burrowing downward at one side of the incision. In three hours it was entirely buried under the skin, with the exception of the small posterior end, which was still visible. The following day the larva was out of sight and the incision healed over except for a small raised

area in the center. In this area a minute round crater-like opening remained. The serous fluid evacuated by the larva was being ejected through this opening. This small raised area slowly increased day by day into a swelling about three-fourths of an inch long, and over one-half inch in diameter and emitted an offensive odor. On April 7, thirty-eight days later, the guinea-pig died. Within five hours after the death of the pig the larva emerged through the small opening. It was 20 mm. in length and 6 mm. in diameter, and we were able to identify it as *D. hominis*. The larva died before pupating.

A few months later Dr. Clark became infested a second time while hunting near Arraijan, on the west side of the Canal. This infestation was also preceded by a tick attachment. On the evening of May 13, after returning from a day's hunt, a small tick evidently a nymph, was found attached on the right side of the abdomen. It was about midway between the groin and the umbilicus, and well beneath the belt line. After the removal of the tick the point of attachment was given the customary treatment of iodin and alcohol for several days without getting healing results. On May 23, a slight pricking sensation was felt in the small lesion. This pricking sensation gradually increased and the small serum-oozing crater could be plainly viewed; the larva inside was easily discernible by the use of a hand lens. The larva was allowed to remain in situ until June 4. An incision was then made and a Dermatobia larva, 2 mm. in length, was removed. This made five larvae of Dermatobia in which a tick had preceded the larva in each instance. Each larva was found in the site of a tick bite, and four of these locations were well protected by clothing.

In order that due weight may be given to the observations it must be remarked that Dr. Clark is a very close observer. He is well versed in medical entomology and is also familiar with the larvae of Dermatobia and the existing theories regarding the manner of disposal of the eggs.

While on the trip mosquito bars were always used while sleeping and due caution exercised while bathing, and the body protected with clothing at all times when possible. In view of these facts it would hardly have been possible for mosquitoes to reach the parts of the body where four of the larvae were situated. However, as has been shown, ticks may easily gain access to the inside of the clothing and attach themselves to almost any part of the body before being discovered.

In these cases the daily application of iodin had no appreciable effect on the young larvae. It did assist, however, in definitely marking the site of each bite, and in proving that each larva was located in a lesion primarily caused by a tick.

In the low lying territory adjacent to Gatun Lake between Gamboa and the quick waters of the Chagras ticks and mosquitoes were found equally numerous. Here the inhabitants were few and no cases of infestation with larvae of Dermatobia were noted among either man or animals. The river regions in the vicinity of the lower reaches of the Chagras and Pequeni rivers, between Alhajuela and Boca Culebra, were well populated and several cases of Dermatobia infestations were observed in domestic animals. Among the animals found to be infested, the dogs and calves seemed to be the most prolific hosts. It is needless to state that both of these animals also act as hosts for numbers of ticks. In this locality both ticks and mosquitoes were present in large numbers. As the party advanced along the Pequeni and Boqueron rivers to the higher regions the inhabitants became fewer in number. At the headwaters of the Boqueron the final camp was made, and some of the surrounding country hunted over and inspected. No inhabitants or domestic animals were found in this vicinity, but the ticks were encountered in greater abundance than in the lower regions, and were found in numbers swarming over the grass and bushes, evidently living on the wild animals such as deer, peccary, tapir, jaguar, puma, ocelot, anteater, capybara, agouti, monkey and many smaller animals with which the region abounded. In this area the higher elevation affording better drainage, the coolness of the nights, due to the high altitude, evidently proved unfavorable for mosquito breeding, as none were found at this place. No signs of Dermatobia larvae were observed in any of the wild animals killed in this locality, but it was at this point that Dr. Clark became infested. Lastly it is worthy of mention that of the many specimens of mosquitoes collected by the party and brought back for identification, not a single specimen of *P. lutzii* was present.

It is to be regretted that none of the five ticks that are suspected of carrying the eggs were preserved. But as these individuals were only a small part of the number that attached themselves to the different members of the party while on this trip, it is not strange that they were not preserved, as no particular attention was paid to these specimens until the lesions caused apparently by their bites failed to heal. However, other specimens found attached to different members of the party, as well as some of those that were found to be so numerous on the grass, bushes, fallen trees, etc., were brought back to the laboratory and all proved to be specimens of the Cayenne tick, *Amblyomma cajennense*. This tick has a variety of hosts, and attacks man and all classes of both domestic and wild animals with equal freedom.

In the face of this evidence it is but reasonable to consider that a species of tick, probably *A. cajennense,* not only acted as the carrier of the eggs, but was also instrumental in assisting the larvae to penetrate the skin.

The incrimination of the tick opens up a new theory on this subject and we hope it will stimulate investigations along a new line on the habits of this insect.

NEW GREGARINES FROM COLEOPTERA

Minnie Watson Kamm

The following pages contain descriptions of two species of gregarines which are believed to be new to literature.

GREGARINA PLATYDEMA nov. spec. (Figs. 1 to 4)

Host: *Platydema excavatum* Say (Tenebrionidae) Det. Chas. A. Hart
Location: Urbana, Illinois, June, 1917
Habitat: Intestine

The sporonts of this species are regularly biassociative, altho anomalies occur more frequently than in any other species observed by the writer. The specimens found occurred in the intestine of a tiny black tenebrionid beetle, about twelve associations and half as many cephalonts being found in each of two hosts. The maximum length of an association found was 2.41 mm.

The individual sporont is cylindrical and slender (Fig. 1), being on an average eight times as long as wide in the primite, the first member of the association, and four times as long as wide in the second member, the satellite. The protomerite of the primite is globular in shape, flattened slightly at its attachment to the deutomerite; its width and height are very nearly identical. The average ratio of LP : TL (see table at end) is about 1 : 12. The deutomerite is slightly constricted at its junction with the protomerite, but soon attains its maximum width which is maintained throughout the entire length, the posterior end being abruptly truncated.

The satellite differs considerably in form from the primite. The protomerite is much flattened, being only one-third to one-half as long as it is wide; it is more flattened in the large than in the small sporonts and is cupped deeply to insure a firm connection between the two members of the association. The deutomerite here is also cylindrical, bluntly rounded posteriorly. The average ratio of LP : TL is about 1 : 15. The width of the protomerite in the satellite bears about the same relation to the width of the deutomerite that it does in the primite: viz., 1 : 1.5.

Each sporont of the association is nearly transparent, no large scattered dark gray granules characterizing this species as is often true of practically transparent species.

The nucleus is conspicuous *in vivo* in both primite and satellite; it is a large sphere situated generally slightly below the center of the deutomerite and in the primite often attains a diameter of very little

* Contributions from the Zoological Laboratory of the University of Illinois, No. 117.

less than that of the sporont itself. In the satellite it is generally smaller in proportion to the diameter of the deutomerite than in the primite. One large karyosome is visible within.

Cephalonts (Fig. 3) were numerous in my material. They are stout-bodied, relatively short and broad, and the ratio of LP : TL (without the epimerite) is about 1 : 5, while that of WP : WD is about 1 : 1. The epimerite consists of a simple cone of about the same length as width surmounting the typically-shaped protomerite. This

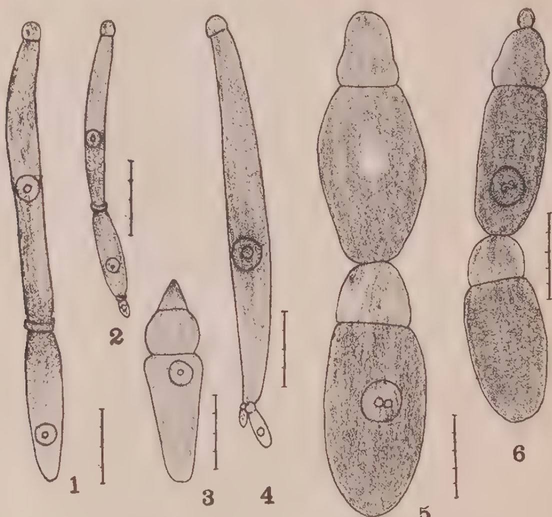

Fig. 1.—Typical association of sporonts of *Gregarina platydema.*

Fig. 2.—Atypical association, *Gregarina platydema,* consisting of a chain of three sporonts, the third minute.

Fig. 3.—Cephalont of *Gregarina platydema.*

Fig. 4.—Three atypically conjoined sporonts of *Gregarina platydema,* the satellites minute.

Fig. 5.—*Gregarina diabrotica,* characteristic association.

Fig. 6.—*Gregarina diabrotica,* abnormal group, the primite of which has not yet lost its epimerite.

In Figures 1, 2, 3 and 4 the reference line is 0.2 mm. long, and in Figures 5 and 6 it is 0.05 mm. long.

cone-shaped epimerite is unusual for the genus gregarina in which I place it, but similar epimerites have heretofore been reported; viz. in *Gregarina statirae* Frenzel in which it is a short cylindrical papilla rounded at the apex, and in *Gregarina acuta* (Léger) in which it is reported as a sharp point (Watson, 1916: 177).

The chief peculiarity which occurs among the sporonts of this species is the great difference in the lengths of the associative sporonts, some being four times longer than others, all, however, perfectly

joined. This variation is not sudden for fairly even gradations occur, and for this reason the presence of a dimorphism cannot be entertained; the sporonts merely become associative long before they are ready for reproduction. All the free cephalonts seen were as large as many of the associative primites and when they would have attained the proportions of mature sporonts would be among the largest measured; it is, of course, true that smaller cephalonts are found embedded in the intestinal epithelium.

In one instance (Fig. 2), an association consisted of three individuals in a chain, the third being minute — one-fourth the length of the first satellite and one-eighth the length of the primite. Another irregularity (Fig. 4) consisted of a triple association, the two satellites being attached to the posterior end of the primite; both were diminutive, only about one-eighth the length of the primite.

In many species studied by the writer where hundreds of specimens were observed no abnormalities or very exceptional ones have been seen to occur. It is possible that such species as the present one are relatively new parasites to their present hosts and have not yet adjusted themselves to the conditions of parasitism offered by the hosts in question.

This species is placed in the family Gregarinidae since that family alone is characterized in part by associations of individuals with septa; and in the genus Gregarina because of the biassociation character and the shape of the epimerite. The species somewhat resembles in form *Gregarina socialis* Léger, a figure but no description or dimensions of which is given in the original reference (Léger, 1906). The latter species, however, is differentiated a), by possessing a small chromidial body in the protomerite, and b), by existing in associations of as many as ten individuals.

Dimensions in microns of several typical live specimens are given below.

	Primite				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite ...	50	50	90	70	20	30	20	40
Length deutomerite...	570	800	920	1130	240	410	520	1180
Width protomerite....	60	60	90	75	50	60	70	80
Width deutomerite....	90	100	140	170	75	100	120	150
Diameter nucleus.....	35	80	70	120	35	70	60	70
Diameter nucleolus....	...	11	...	40	...	20	...	20
Total length sporont..	620	850	1010	1200	260	440	540	1210
Ratio LP : TL......	1:12.4	1:17	1:11.2	1:17	1:13	1:15	1:27	1:30
Ratio WP : WD......	1:1.5	1:1.6	1:1.5	1:2	1:1.5	1:1.6	1:1.7	1:1.9
Total length association	880	1290	1550	2410				

Numerous biassociative and young solitary sporonts of this species have been taken from each of a half dozen beetles of the species listed which is a pest to both wild and cultivated cucumber vines.

The sporonts are elongate-cylindrical, flattened at each end and about three times as long as the maximum width. The largest sporont seen measured 270μ by 105μ. The largest association was 530μ long. The ratio LP: TL of the primite was about 1: 3.5; that of WP: WD 1: 1.6.

The protomerite of the primite is broadly dome-shaped and constricted somewhat in the mid region. It is slightly longer than wide, the widest portion being in the posterior third. It again becomes constricted at the septum; the whole shape, therefore, is unique and a constant and characteristic feature of the species. The outline of the deutomerite is typical of that of many gregarines, widening immediately below the septum and retaining the same width throughout the entire length, except at the broadly rounded posterior extremity. The protomerite of the satellite is lower and lacks the constriction of that of the primite; it is regularly dome-shaped with the apex slightly flattened at its contact with the primite. The deutomerite is essentially like that of the primite.

The protoplasm is dark gray, almost black in transmitted light in the deutomerite and slightly less dense in the protomerite. The nucleus is spherical, of good size, and contains two or three minute karyosomes.

The epimerite is small, sessile and spherical, characteristic of the genus Gregarina, in which it is placed because of the epimerite and the biassociative sporonts. A table of a few typical measurements in microns of live sporonts follows:

	Primite				Satellite		
	a	b	c	d	a	b	c
Length protomerite	60	70	50	60	50	50	40
Length deutomerite	200	190	100	165	210	200	180
Width protomerite	60	50	40	50	70	80	50
Width deutomerite	90	90	60	70	100	105	65
Total length sporont	260	260	150	225	270	250	220
Ratio LP : TL	1:4.3	1:3.7	1:3	1:3.6	1:5.4	1:5	1:5.5
Ratio WP : WD	1:1.5	1:1.8	1:1.5	1:1.4	1:1.4	1:1.3	1:1.3
Total length association	530	510	370				
Diameter nucleus	...	...	...	30			

GREGARINA DIABROTICA nov. spec. (Figs. 5 and 6)

Host: *Diabrotica vittata* Fabr. (Chrysomelidae)
Location: Urbana, Illinois, June, 1917
Habitat: Intestine

This beetle is also one of the hosts of a nematode, the larvae being found in the body cavity of two specimens in countless hundreds massed tightly against the internal organs. That the larvae exert a

baneful influence upon the host is shown by the fact that after half a dozen of the host beetles had been kept in captivity for twelve hours none showed ill effects except two which were sluggish, apparently at the point of death when opened, and which proved to be heavily parasitized. The hosts must, therefore, eat regularly and often in order to feed so great a number of intruders.

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CENTRORHYNCHUS PINGUIS N. SP. FROM CHINA*

H. J. VanCleave

The Acanthocephala, as a group, have a very broad geographical distribution. Practically every country, the fauna of which has been studied, has revealed considerable numbers of these worms parasitic in the various classes of its vertebrates. In going over the literature upon this group the writer has found no reference of any kind to their occurrence in China. This is probably due to the incompleteness of the published records concerning the fauna of that country and does not necessarily indicate any actual scarcity of Acanthocephala. In 1915 Dr. R. T. Shields sent material from the intestine of a magpie at Nanking, China, to Professor Henry B. Ward, who kindly turned them over to the writer for study. A study of stained whole mounts and of serial sections has demonstrated the fact that these individuals belong to a new species of the genus Centrorhynchus which is described below.

Centrorhynchus pinguis nov. spec.

With the characters of the genus VanCleave (1916-a). Body robust, with anterior half slightly inflated. Entire length about 15 mm.; maximum diameter in anterior third of body, about 2.5 mm.; diameter in posterior attenuated third about 1.2 mm. Proboscis about 0.77 mm. long; region anterior to insertion of proboscis receptacle ovoid, about 0.48 mm. long by 0.38 mm. in diameter; posterior to insertion of receptacle a truncated cone with the base at the line of union with body proper. Proboscis armed with about thirty-two longitudinal rows of about sixteen hooks each. Embryos 48 to 65μ long by 24μ in diameter; elliptical, with the three membranes concentric. Males not observed.

Host: Magpie, in intestine. Locality, Nanking, China. Type female deposited in Parasitological Collection of the University of Illinois; catalog number 18.1. Paratypes in collection of the writer.

The specimens available for this study were preserved in formalin and tho evidently killed in the same reagent, were in splendid histological condition. In toto-mounts the marked translucency of structure characteristic of formalin specimens was of great value in permitting a close examination of internal structures. However,

* Contributions from the Zoological Laboratory of the University of Illinois, No. 121.

some of the finer points of structure were determined from a series of longitudinal sections.

The proboscis receptacle (Fig. 7) is distinctly of the type characteristic for the genus Centrorhynchus. It is a sac shaped structure 1.3 mm. long, inserted near the middle of the prosboscis and gradually diminishing in size toward its posterior extremity where it ends in a small, bluntly rounded termination. The walls of this organ are composed of two concentric layers of muscle of which the outer has a thickness of about 12μ, while the inner layer is two or three times as thick. The large invertors (*ip*) of the proboscis fill practically the entire space within the receptacle. Near the middle of the receptacle the invertors are separated by the brain (*br*), which in this species is an ovoid mass about 0.17 mm. long and 0.07 mm. broad located about 0.4 mm. anterior to the posterior tip of the receptacle. Within the brain the individual ganglion cells are ovoid in shape with a length of 41μ and a breadth of 30μ. The nuclei in these cells are very conspicuous, having a diameter of about 15μ. Fibers from the invertors of the proboscis pass through the wall of the receptacle in the region near its posterior tip and continue through the body cavity as the retractors of the proboscis receptable (*pr*).

The body wall (Fig. 5) presents a type of structure similar to that described by the writer (1916: 170) for Arhythmorhynchus. The high degree of development of the muscle layers (*bm*) gives the body wall in this species a peculiar appearance closely simulating that of a parenchyma. The similarity is heightened by the presence of numerous embryos (*e*) which have found their way from the body cavity proper (*bc*) into the meshwork of this loosely organized tissue. In the paper referred to above, the writer called attention to the similarity existing between the fundamental structure of the muscle cells of nematodes and of members of the genus Arhythmorhynchus. In *Centrorhynchus pinguis* this similarity is even more striking. Figure 6 shows the structure of a single muscle cell taken from a longitudinal section through the body wall. Each such cell is comprised of two distinct regions: a sac of undifferentiated cytoplasm (*cm*), containing the nucleus (*n*); and at the opposite end of the elongated cell a group of differentiated muscle fibrillae (*mf*). In the cells under consideration the fibrillae are restricted in their distribution to the margin of the cells contiguous to the subcuticula. In the muscle cells shown in Figure 5 these cells have been cut in an oblique plane so that only the fibrillar portion of each cell is shown. It should be kept in mind in this connection that the usual arrangement of the body muscle layer in Acanthocephala is such that the nuclei all lie in the dorsal region of the body. Here they occur in two more or less sharply defined

longitudinal rows. In one tangential section through a muscle cell of *C. pinguis* the writer has observed two nuclei within the same muscle cell, lying some distance apart, indicating the possibility that either the muscles of the two sides of the body are derived from large binucleate cells or are possibly the result of a fusion of the cells from the two sides of the body.

Internally, the proboscis shows a differentiation in the structure of its wall which bears a rather direct relationship to the division into anterior and posterior regions separated by the line of insertion of the proboscis receptacle. The external marking off into regions and the internal differences in structure do not, however, coincide, precisely. The wall of the anterior proboscis region is thicker and more distinctly fibrous in structure than is the wall of that part of the proboscis posterior to the insertion of the proboscis receptacle (Fig. 7). In the anterior region conspicuous groups of fibers run across the wall. The association of these fibers with the well developed root processes upon the hooks in the same region indicates a probable greater degree of freedom of movement of the hooks anterior to the insertion of the receptacle than of those in the posterior region of the proboscis.

Hooks upon the proboscis (Fig. 2) are distinctly of two types. Those anterior to the insertion of the receptacle are heavy, with conspicuous reflexed root processes, while those posterior to the insertion are more spine like and rarely possess true root processes. In these latter the basal portion of the hook or spine is embedded in the proboscis wall and alone serves for connection with that organ. The largest hooks upon the proboscis are strongly recurved, 53μ long, and with a diameter of 18μ at the point where they emerge from the proboscis wall.

The female genital tract is, in most individuals, completely obscured by the accumulation of embryos in the posterior body region. One female, stained and mounted in toto, gave a very clear view of the relations of the parts of the female genital organs as shown in Figure 4. The embryos in this species (Fig. 3) while covered with fully formed membranes display considerable degree of variability in size.

SUMMARY

The description of *Centrorhynchus pinguis,* nov. spec. from the intestine of a magpie from China furnishes apparently the first record of the occurrence of Acanthocephala in China. In discussing the morphology of *C. pinguis* especial attention is given to the cellular elements of the body musculature.

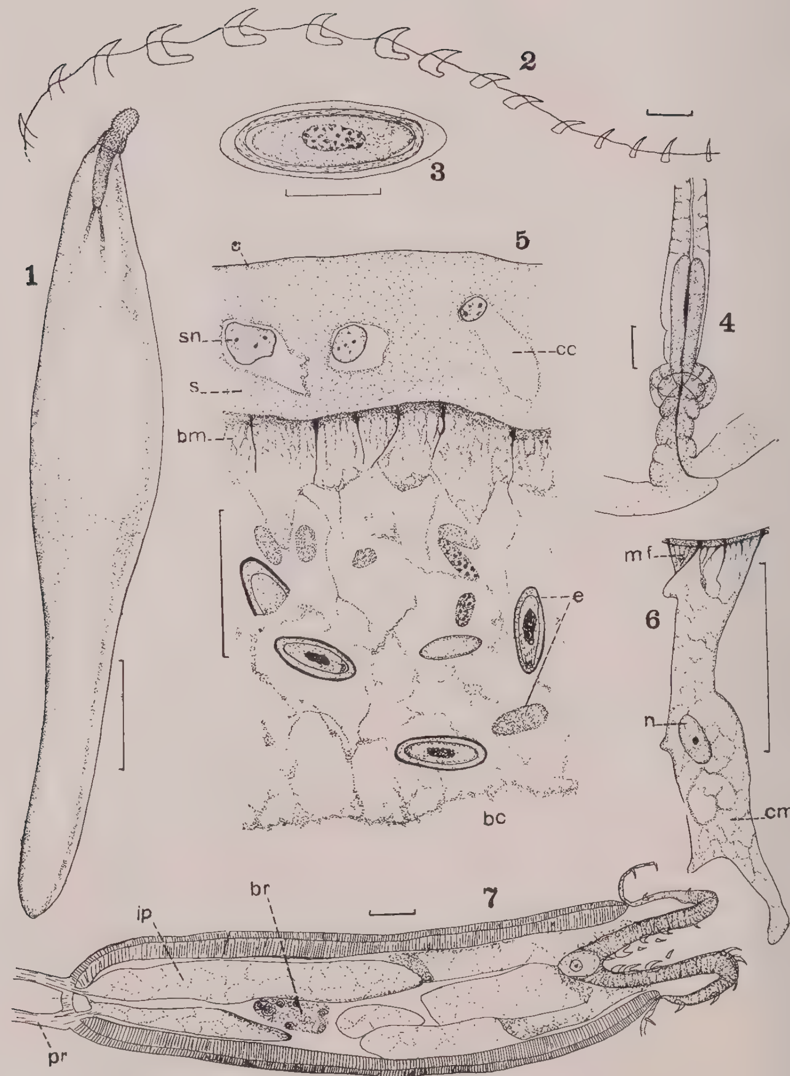

PLATE III

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Explanation of Plate

All drawings were made with the aid of a camera lucida. The magnification of each figure is indicated by the reference line accompanying it which has the value of 0.1 mm., except that in Figure 1, it has the value of 2 mm., and in Figure 3 of 0.02 mm.

Morphology of *Centrorhynchus pinguis* nov. spec.

Fig. 1.—Type female showing general body form. From toto mount stained in Ehrlich's acid hematoxylin and mounted in damar.

Fig. 2.—Profile of proboscis of type female, showing a single longitudinal row of hooks.

Fig. 3.—Embryo from body cavity of female. Drawn from a longitudinal section of body.

Fig. 4.—Genital tract of female, from toto mount. The anterior end, including the selective apparatus, hidden from view in specimen by accumulation of embryos within body cavity.

Fig. 5.—A portion of body wall from longitudinal section. *bc,* body cavity; *c,* cuticula; *cc,* circular canal of lacunar system; *bm,* body musculature; *e,* embryo; *s,* subcuticula; *sn,* subcuticular nucleus.

Fig. 6.—A single muscle cell, drawn from longitudinal section of body wall. *cm,* undifferentiated cytoplasmic mass; *mf,* muscle fibrillae; *n,* nucleus.

Fig. 7.—Section through inverted proboscis and proboscis receptacle. *br,* brain; *ip,* invertor of proboscis; *pr,* retractor of proboscis receptacle.

A NOTE ON THE CULTIVATION OF *TRICHOMONAS INTESTINALIS*

Mark F. Boyd
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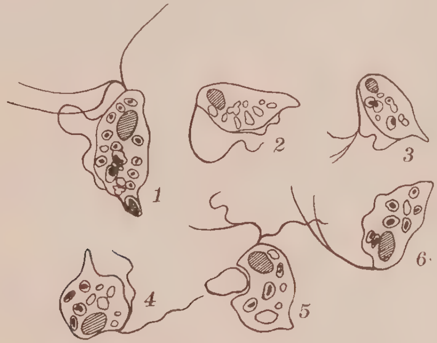
In 1915 Lynch reported the cultivation of a trichomonad secured from the gums and vagina of a negress suffering from an acute gingivitis and catarrhal vaginitis, while in 1917 Ohira and Noguchi report the cultivation of trichomonads from the dental tartar. So far as I know, no report of the cultivation of these flagellates from the intestinal tract has been made.

In making a parasitological examination of the large and small intestine of a white male removed at autopsy, the colon was found to contain a considerable quantity of very soft, light yellow fecal material. Microscopical examination of this fecal material demonstrated not over two trichomonads per slide, each slide being prepared from a single loopful of feces. Loopfuls of feces were transferred to tubes of physiological saline, acid broth (plus 1) and neutral broth, both of the latter being made from meat extract.

After three days incubation at 37° C., the supernatant fluid and sediment of bacteria and fecal debris was examined for the trichomonads. None were found in any of the inoculations made into broth, while in the sediment of the inoculations made into saline, several trichomonads were found in each field, the numbers being markedly increased over those observed in the feces from which the inoculations were made. After ten days growth, transfers of single loopfuls of sediment were made to fresh tubes of unsterilized fecal suspensions in saline, the feces employed being from a person free from this flagellate. By this time none of the broth tubes had shown a growth. After the second transfer the numbers of flagellates continued to increase, as many as a dozen being observable in a single microscopic field. After eighteen days in the second culture they were still abundant in the sediment and transfers were made to another series of unsterilized fecal suspensions. Uninoculated controls of this fecal suspension were also incubated. In the third transfer the organisms have multiplied extensively, but are apparently not as numerous as in the second transfer. The cultures are now in the fourteenth day of the third transfer, and the trichomonads are still abundant. No flagellates have been found in the control tube.

A number of the trichomonads are shown in the accompanying illustration, which was made from films fixed in sublimate alcohol and stained with Heidenhain's iron hematoxylin. Before smearing the organisms were concentrated and mixed with dilute serum water to insure fixation. Well fixed individuals show a rather elongated, pear-shaped body, blunt at the anterior extremity, pointed at the posterior. The large nucleus is visible at the anterior end, while the body cavity is filled with vacuoles containing bacteria in various stages of digestion. In well spread individuals three flagella are observable arising from the anterior end, while from the same situation arises an undulating membrane which extends towards the posterior extremity, but apparently does not reach to the tip. An axostyle has not been observed. Individuals vary in length from 12



Group of *Trichomonas intestinalis*

to 18μ and in breadth from 6 to 9μ, depending largely on the position in which fixed. These characteristics indicate the organism probably belongs to the genus Trichomonas, although the absence of an axostyle does not confirm this diagnosis. If the failure to observe an axostyle is due to technical errors, the species is undoubtedly *T. intestinalis*.

To date in none of the cultures have I observed cysts of any character, so that the conflicting observations of Wenyon (1910) and those of Lynch (1916) upon cyst formation in this species cannot be reconciled.

In the case from which these trichomonads were derived the autopsy revealed an aortic insufficiency, a diffuse arteriosclerosis of the kidneys, to which death was due, and a diffuse, pseudo-membranous colitis, extending from the ilio-cecal valve to the rectum.

From these observations it would appear that the recognition of intestinal flagellates might be facilitated by incubating fecal suspen-

sions in saline for several days before examining, rather than by the direct examination of fresh feces. This procedure might prove of value should it be ascertained these organisms possess pathological significance.

I shall attempt the isolation and propagation of this flagellate in pure culture with a suspension of a single bacterial species as food. It would appear that such a combination would be necessary since they appear to require the ingestion and intracellular digestion of micro-organisms for nourishment.

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ADAPTABILITY OF SCHISTOSOME LARVAE TO NEW HOSTS

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The spread of any digenetic trematode is limited by the distribution of the molluscs which serve as its intermediate host. Trematode species which can become adapted for development to several different species of intermediate hosts have a much better chance of entering new localities than those which are absolutely specific in one mollusc. The literature on the cercariae shows a number of species which can develop equally well in different species and even different genera of molluscs. The best known example of the relation of adaptability in intermediate hosts to the spreading of a digenetic trematode to new localities is the sheep liver fluke, *Fasciola hepatica,* which has become widely distributed to various parts of the world, adapting itself to some species of the snail genus Lymnaea found in each new region.

Although the larval stages of the human blood flukes have been known less than five years, the records already show a surprising lack of specificity in intermediate hosts. Leiper (1916: 411) records the cercaria of *Schistosoma haematobium* from Egypt in *Bullinus contortus* and *Bullinus dybowski,* and the cercaria of *Schistosoma mansoni* from *Planorbis boissyi.* Cawston (1917: 133) records the cercaria of *Schistosoma haematobium* in South Africa from *Physopsis africana,* and in Venezuela Iturbe and Gonzalez (1917) found the cercaria of *Schistosoma mansoni* in *Planorbis guadelupensis* Sowerby. The cercaria of *Schistosoma japonicum* has so far been described from only one host, the katayama snail, *Blanfordia nosophora.* It is significant that this species is an operculate snail belonging to a different order of the Gastropoda from the intermediate hosts of *S. haematobium* and *S. mansoni.* In addition to these at least two of the species of forked-tailed cercaria develop as described by Faust (1915: 122 and 1918: 105) in more than one species of intermediate host. *Cercaria gracillima* is described from *Lymnaea proxima* Lea and *Physa gyrina* Say from the Bitter Root Valley, Montana, and *Cercaria gigas* from *Planorbis trivolvis* Say and *Physa gyrina* Say from Illinois.

My own studies on the forked-tailed cercariae from the United States have shown several striking examples of lack of specificity in the choice of intermediate host. *Cercaria douthitti* Cort which was described (1915: 49) from *Lymnaea reflexa* Say taken near Chicago,

Illinois, was later found in the region of Douglas Lake, Michigan, in *Lymnaea stagnalis oppressa* (Say), *Lymnaea stagnalis perampla* Walker and *Physa ancillaria parkeri* (Cuvier). *Cercaria douglasi* Cort was found in the same region in species of snails belonging to two different genera, viz., *Physa ancillaria* Say, and *Lymnaea emarginata angulata* (Sowerby). A third species of forked-tailed cercaria, as yet undescribed, was found in a single small beach pool on the shore of Douglas Lake in species belonging to three different genera of snails, viz., *Planorbis trivolvis* Say, *Lymnaea exilis* Lea, and *Physa ancillaria* Say.

The data given above seems to clearly indicate that the forked-tailed cercariae readily adapt themselves to new molluscan intermediate hosts. Further studies on the intermediate hosts of the human schistosomes will undoubtedly add to the list of snails which can be utilized as intermediate hosts by these species. The striking dissimilarity between *Blanfordia nosophora,* the intermediate host of *Schistosoma japonicum,* and the intermediate hosts of *Schistosoma haematobium* and *S. mansoni* is also very significant in this connection. If there were any great degree of specificity in the intermediate hosts among these forms, species of the same genus would hardly be expected to develop in intermediate hosts so entirely unrelated. The close relationship of *Cercaria douthitti* and *Cercaria douglasi* to the human schistosomes, indicated in a previous publication (Cort, 1917), also makes the adaptability of these species to a variety of intermediate hosts significant in relation to specificity in the human forms.

Since the cercariae of the human schistosomes penetrate directly into their host, and can develop to maturity in rats, cats, dogs and cattle, as well as man, they will probably spread rapidly if carried into any region where suitable intermediate hosts are found. It is known from case records and records of the immigration stations that *Schistosoma japonicum* has been brought into the United States from the Orient. Before August, 1917, when schistosomiasis was placed on the exclusion list by the Surgeon-General of the United States Public Health Service, orientals with this disease are known to have entered this country in considerable numbers. In many of the irrigated regions of the Pacific Coast states, oriental laborers from countries in which schistosomiasis is prevalent live in much the same relation to the soil as in their own country, making ideal conditions for the spread of this disease provided a type of snail in which the flukes can develop is present. It is, therefore, evident that the question of the adaptability of the schistosomes to new intermediate hosts becomes a problem of great significance in relation to the possible

spread of this disease in the United States, and it is of great importance to discover whether there are snails in this country in which the blood flukes can develop.

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ON THE PARASITISM OF CARBONIFEROUS CRINOIDS *

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The nature of the evidences of parasitism in geological epochs will doubtless be of interest to all parasitologists. No statement concerning parasitic conditions among fossil animals is made in any of the usual text-books of paleontology, and the general reference works of zoology make no mention of the matter. Abel (1912), however, in his excellent work devotes two paragraphs to a review of possible conditions of parasitism among fossil animals, calling attention especially to the work of von Graff on the swollen stems of Carboniferous crinoids of Germany. Stromer von Reichenbach (1909) refers to parasitism among fossil corals, and figures a cross section of *Pleurodictyum problematicum* from the Lower Devonian of Eifel. This is regarded by Abel as an example of symbiosis. It seems quite probable that Abel is correct in his interpretation.

Robert Etheridge (1880) was the student who first recognized the nature of the swollen stems of fossil crinoids, though he was unable to determine the nature of the parasite. This was later accomplished by L. von Graff (1885), who was able to determine the nature of the parasite, having discovered the carbonized remains of one of the myzostomids which he regarded as the infecting form. Graff reviewed the literature and referred to numerous species of crinoids which showed swollen stems, some of the species having been based on these swollen stems, which were mistaken for calyces. Graff compared very carefully his results with the swollen crinoid stems as described for recent forms in the Challenger reports, where the infecting forms were known to be myzostomids.

John M. Clarke (1908) has written an excellent paper on the pre-carboniferous evidences of communism and commensalism, calling his study "The Beginnings of Dependent Life." In his extensive collections he has found no trace of definite parasitism, but certainly the cases described by him may be regarded as the beginnings of parasitism. It seems probable at present that true parasitism did not begin until the Carboniferous Period.

Swollen stems of crinoids have often been seen by paleontologists both in America and in Europe, but few have recognized their para-

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* An abstract of this paper was published in the Proceedings of the American Society of Zoologists, Dec. 27, 1917, p. 34.

sitic nature. A few species and genera of fossil crinoids have been based on the enlarged stems, the specimens being regarded as aberrant calyces.

The specimens of crinoid stems at the writer's disposal are the first to be recognized in America as many ways suggesting parasitism. Specimens are fairly common in collections of fossil invertebrates, and especially so from the Keokuk beds, where the swollen stems often assume a geoditic nature, which, owing to complete mineralizaztion, destroys the anatomical details and leaves only the outward form.

There is nothing to be added to what is already known concerning the parasitism of Carboniferous crinoids, save that this is the first record made of swollen crinoid stems in America. They have frequently been seen, but so far as I can determine, their nature has never been recognized. There is so little difference between the American specimens and those described by von Graff and Etheridge that a very brief description will suffice.

The specimens vary from a half inch to four inches in maximum diameter, the plates of the stems being enlarged and spread apart. The columnars are often spread out to four or five times their normal diameter, the space between the series of columnars being widened to several millimeters. The individual plates are not separated. The enlargements are often mere bulgings in the stem, and again they take the appearance of large tumors, tapering at each end to join the stem. It is impossible in the present case to determine the location of the parasite, but Graff found the parasite located near the point of greatest enlargement of the stem. The swelling of the stem usually does not distort its pentagonal symmetry.

It should be noted here that Bassler (1908) has described objects of a similar nature, and has interpreted them as due to the geodization of the fragment of stem. The objects I have studied are, however, entirely different from the specimens studied by Bassler, judging from his figures and descriptions. There can be no doubt that many enlarged crinoid stems do not represent parasitism, but are the result of the formation of the geode. Many of them, however, may represent parasitism, and paleontologists have not, to date, taken this fact seriously into consideration.

The writer's interest in these objects is due to the fact that the swollen stems must be regarded as the first evidences of disease in geological history. So far as known no fossil animals suffered from disease prior to the Carboniferous, and these tumor-like masses in the stems of crinoids must be regarded as the earliest evidences of pathological processes. Diseased conditions became more and more apparent from the Carboniferous Period down to the present and disease is more prevalent today than ever before in the history of the world.

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REVIEW AND NOTES

Infection and Resistance. Hans Zinnser, M.D. With a chapter on Colloids and Colloidal Reactions by Prof. Stewart W. Young. Second edition, revised. The Macmillan Company, 1918. xiii + 585 pages. $4.25.

The second edition of this admirable and scholarly work was prepared, as the author says, under difficult conditions—far from the facilities of libraries and files of reprints. Nevertheless, the changes represent fairly the advances in the field of knowledge since the first edition was completed.

To the chapter on anaphylaxis has been added a wealth of new material. The section on infection and immunity in poliomyelitis is entirely new, as also an extended discussion of immunity in syphilis. A whole chapter has been added on serum enzymes, leukocytic enzymes, on the physical factors which enter into serum reactions, and on colloidal gold reaction. The book has appealed, and will continue to appeal, to those who are looking for insight into the fundamental principles on which rests our knowledge of infectious diseases. Every student of medicine should know and use this work in preparation for the handling of cases in clinic or laboratory.

NOTES

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